Research Article

Hanem G. Abd Elrahman, Abeer Ramadan*, Eman M. Saleh, Alia A. Saleh, Noha A. Sabry and Mohamed R. Mohamed

Association study of polymorphisms in ABCA7, clusterin, and MS4A6A genes with Alzheimer's disease in the Egyptian population

[Mısır Halkında Alzheimer Hastalığı ile ABCA7, Clusterin ve MS4A6A Genlerinde Polimorfizmlerin İlişkisi]

https://doi.org/10.1515/tjb-2020-0135
Received September 5, 2019; accepted October 1, 2020; published online November 30, 2020

Abstract

Alzheimer's disease is a genetically heterogeneous brain disease which is associated with a decrease in thinking capacity and changes in personality. Various gene polymorphisms could be associated with the occurrence of Alzheimer's disease.

Objectives: This study aimed at exploring the association of ABCA7 rs3764650, CLU rs11136000, and MS4A6A rs610932 genetic variants with AD in a sample of the Egyptian population.

Methods: DNA extraction was done from peripheral blood. Genotyping was done using PCR followed by restriction fragment length polymorphism (RFLP) in 100 Alzheimer patients of age 60 years old or above and 100 cognitively normal controls. DNA sequencing was done for eight samples with different genotypes to confirm the results.

Results: No significant difference was found in genotypic or allelic distribution of CLU rs11136000 between patients and controls. the frequency of CLU CC genotype among AD patients, was lower in Apo E 4 carriers compared to Apo E 4 non-carriers with a significant difference (p=0.003). No significant difference was found in genotypic or allelic distribution of ABCA7 rs3764650 and MS4A6A rs610932 between patients and controls.

Conclusion: We concluded that AD not associated with Clustrin rs11136000, ABCA7 rs3764650 and MS4A6A rs610932 genes polymorphism.

Keywords: ABCA7; Alzheimer's disease; clusterin; MS4A6A; polymorphisms.

Öz

Giriş: Alzheimer Hastalığı, düşünme kapasitesindeki azalma ve kişilikteki değişikliklerle ilişkili genetik olarak heterojen bir beyin hastalığıdır. Alzheimer hastalığının ortaya çıkmasıyla çeşitli gen polimorfizmleri ilişkilendirilebilir.

Amaç: Bu çalışma, Mısır popülasyonunun bir örneğinde ABCA7 rs3764650, CLU rs11136000 ve MS4A6A rs610932 genetik varyantlarının AD ile ilişkisini araştırmayı amaçlamaktadır.

Yöntemler: Periferik kandan DNA ekstraksiyonu yapıldı. Genotipleme, PCR kullanılarak yapıldı ve ardından 60 yaş ve üzerindeki 100 Alzheimer hastasında ve 100 bilişsel olarak normal kontrolde kısıtlama fragman uzunluğu polimorfizmi (RFLP) yapıldı. Sonuçları doğrulamak için farklı genotiplere sahip sekiz numune için DNA sıralaması yapıldı.

***Corresponding author: Dr. Abeer Ramadan Salamah**, Molecular Genetics and Enzymology Department, Human Genetic and Genome Research Division, National Research Centre, Cairo, Egypt, E-mail: dr_aramadan1978@yahoo.com. https://orcid.org/0000-0001-5757-1769

Hanem G. Abd Elrahman, Eman M. Saleh and Mohamed R. Mohamed, Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

Alia A. Saleh and Noha A. Sabry, Psychiatry Department, Faculty of Medicine, Cairo University, Cairo, Egypt

Bulgular: CLU rs11136000'in genotipik veya allelik dağılımında hastalar ve kontroller arasında anlamlı bir fark bulunmadı. AD hastaları arasında CLU CC genotip sıklığı, Apo E 4 taşıyıcılarında, Apo E 4 taşıyıcı olmayanlara kıyasla anlamlı bir farkla daha düşüktü (P = 0.003). Hastalar ve kontroller arasında ABCA7 rs3764650 ve MS4A6A rs610932'nin genotipik veya allelik dağılımında anlamlı bir fark bulunmadı.
Sonuç: AD'nin Clustrin rs11136000, ABCA7 rs3764650 ve MS4A6A rs610932 gen polimorfizmi ile ilişkili olmadığı sonucuna vardık.

Introduction

Alzheimer's disease (AD) is a progressive, cognitive disability neurodegenerative condition. The brain of an AD patient has a bounty of plaques and tangles. Plaques are stores of a protein fragments called beta amyloid (Aβ) which develop in the spaces between nerve cells. Tangles are twisted filaments of another protein called tau protein which build up inside the cells [1].

AD prevalence fluctuates according to various factors including: age, inheritance, comorbidities, and level of education. Due to the difficulty of AD early diagnosis, the need for developing clinically applicable molecular biomarkers for its diagnosis is growing rapidly. Accurate blood-based biomarkers of AD could be an early diagnosis tools for this overwhelming neurodegenerative disease that is simple and noninvasive [2].

There are numerous hereditary loci which adjust AD hazard. Apolipoprotein E 4 (Apo E4) is the major identified risk factor for AD. Genome-Wide Association Studies (GWAS) identified other genetic loci markers for AD risk including rs3764650 polymorphism in *ABCA7* gene, rs11136000 polymorphism in Clusterin gene, and rs610932 polymorphism in *MS4A6A* gene. These polymorphisms are on the "AlzGene Top Results" list which outlines the most settled genes related to AD [3].

ATP-binding cassette transporter A7 (*ABCA7*) gene is situated on chromosome 19p13.3 and consists of 47 exons. *ABCA7* protein is involved in the regulation of Aβ homeostasis in the brain as its inadequacy encourages Aβ production by expanding the levels of β-secretase 1 in neurons. *ABCA7* has also been related with amyloid plaques in human neurons, recommending its possible involvement in AD pathogenesis [4].

Clusterin (*CLU*) gene is situated on the human chromosome 8p21.1. It is composed of nine exons and encodes a 70-kDa multifunctional glycoprotein called CLU which is also known as ApoJ. *CLU* is speculated to act as an extracellular chaperone that is involved in the removal of misfolded proteins and apoptosis regulation. Moreover, it has a significant role in lipid transport, complement regulation, and membrane protection. *CLU* has also protective role against AD by preventing Aβ aggregation and promoting its removal from the brain via low-density lipoprotein-related protein 2 (LRP2) across the blood–brain barrier [5].

Membrane-Spanning 4-domains, subfamily A (*MS4A*) gene is situated on chromosome 11q12.2. It encodes a member of the membrane-spanning 4A gene family. Members of this protein family are described by similar structural characteristics and intron/exon splice boundaries that are comparative. They show one of a kind expression patterns among non-lymphoid tissues and hematopoietic cells. Moreover, they are vital to immune cell survival and activation as they work as accessory proteins which encode receptor or ion channel functions [6].

As genetics for AD in the Egyptian population need to be explored, the aim of the current study was to investigate the association of *ABCA7* rs3764650, *CLU* rs11136000, and *MS4A6A* rs610932 genetic variants with AD in a sample of Egyptian patients.

Subjects and methods

Study design and population

The study was carried out on 100 Egyptian AD patients (60 years or older) and 100 age- and sex-matched controls. The characteristics of the studied groups are presented in (Table 1). The research proposal is approved by the National Research Centre ethical committee, Cairo, Egypt (Approval Number: 18135). A written informed consent was obtained from all participants prior to clinical investigations.

AD patients were attending the Old Age Psychiatry Unit, Faculty of Medicine, Cairo University. All patients fulfilled the diagnostic criteria of Major Neurocognitive Disorder due to AD according to the Statistical Manual of Mental Disorders (DSM-5). The Washington University Clinical Dementia Rating Scale (CDR) - assignment algorithm was used for AD assessment and all patients scored 1 or more/3. Mini Mental State Examination (MMSE) and MoCA-Basic were used for further confirmation [7].

The healthy controls included in this study were volunteers recruited from Geriatric Social Centre, Cairo University. They were needed to attain a normal score in both MMSE and CDR Scale.

Exclusion criteria of this study were having dementia because of other causes than AD such as mixed pathology

Table 1: Characteristics of the studied groups.

Descriptive data	Control n=100	Cases n=100	p-Value
Age	65.9 ± 5.02	70.7 ± 7.5	0.1
Gender			
Female	76 (76%)	55 (55%)	0.02
Male	24 (24%)	45 (45%)	
Education	96 (96%)	40 (40%)	0.01*
Residence			
Rural	7 (7%)	47 (47%)	0.02
Urban	93 (93%)	53 (53%)	
Family history AD			
Negative	95 (95%)	87 (87%)	0.02
Positive	5 (5%)	23 (23%)	
Clinical dementia rating scale (CDRS)			
Negative	100%	0%	0.001**
Nearly mild	0%	0%	NA
Mild	0%	36%	0.001**
Moderate	0%	49%	0.001**
Sever	0%	15%	0.001**
Montreal cognitive examination basic (MoCA)	25.4 ± 3.07	9.4 ± 5.4	0.001**
Mini-mental state examination (MMSE)	29.07 ± 1.2	16.7 ± 6.8	0.001**

Age, MMSE and MoCA are represented as Mean ± SD, while CDRS, gender, education, residence are represented as number and percent. *p-Value ≤ 0.05 significant; **p value ≤ 0.01 highly significant.

or comorbid mental and medical condition in addition to utilizing drugs or any substance which may meddle with subjective testing.

Molecular tests

The SNPs information for ABCA7 rs3764650 (NC_000019.10:g.1046521T>G), Clustrin rs11136000 (NC_000008.10:-g.27464519T>C), and MS4A6A rs610932 (NM_001330275.1:c.*149+175A>C) were obtained from the NCBI (http://www.ncbi.nlm.nih.gov/snp/) and ensemble databases (https://www.ensembl.org/Homo_sapiens/Info/Index).

RFLP

Genotyping of polymorphisms was done by PCR using the Bio-Rad T100TM Thermal Cycle followed by enzyme digestion with the suitable restriction enzyme.

DNA was extracted from peripheral blood (in EDTA-containing vacutainers) by utilizing DNA extraction kit (Qiagen) catalogue number 51304 as per the manufacturer's protocol. Nanodropper 2000 (Thermo-Scientific) was used to estimate the DNA concentration and purity (DNA absorbance ratio at 260 nm to absorbance at 280 nm (A260/A280) was considered).

Target sequences gene were amplified using specific primers. The sequences of forward and reverse primers are shown in (Table 2). Primer sequences were checked and revised using primer blast database (http://www.ncbi.nlm.nih.gov/BLAST). The primer selection was done according to the GENBANK database.

PCR amplification was done in a total volume of 30 µL consisting of 100 ng of each DNA sample, 1X QIAGEN Taq buffer, 0.2 mmol/L of dNTPs, 0.5 U QIAGEN Taq DNA polymerase, and 10 pmol each of the specific primers.

For ABCA7 rs3764650 SNP, the PCR reaction condition was 95 °C for 5 min, followed by 30 cycles of (95 °C for 30s, 56 °C for 45s, 72 °C for 30s), and then a final extension at 72 °C of 10 min. For Clusterin rs11136000 and MS4A6A rs610932 SNPs, the PCR reaction condition was 95 °C for 5 min, followed by 30 cycles of (95 °C for 45s, 52 °C for 45s, 72 °C for 60s), and then a final extension at 72 °C of 10 min.

The products of amplification were analyzed by 2% agarose gel electrophoresis (along with 100 bp DNA ladder gene ruler (Catalog number: SM0241). The gel is stained with ethidium bromide.

Enzyme digestion was done by incubation of 10 µL of the PCR product in 2 µL of 10X buffer with 1–2 µL of the appropriate restriction enzyme at 37 °C overnight.

The digested PCR products were electrophoresed at 120 V for 1/2 h on 3% agarose gel. In order to examine the digested PCR fragments in the gel, we used a Gel

Table 2: Primers sequence used in the PCR amplification for the polymorphic loci.

SNP	Primer sequence (5′-3′)	PCR size	Annealing	Re recognition site	Allele	Product (bp)	Ref
ABCA7	F:5′ATCCGTGCTATGTGGACGAC 3′	642 bp	56 °C	HpyCH4III	G	354, 136 bp	[8]
rs3764650	R:5′ACCTTGAGCACCAGAACCAG 3′			5′…ACN↓GT…3	T	490, 152 bp	
CLU	F:5′-CAGATTCTTACAGAAGGATTGG-3′	389 bp	52 °C	MluCI	C	222, 167 bp	[9]
rs11136000	R:5′-TTGCTCCAGTTCAAGCTTC-3′			5′…↓AATT … 3	T	222, 120 bp, 47 bp	
MS4A6A	F:5′-CCCAGAAA CATTTCCCAGAA-3′	215 bp	52 °C	NIaIII	A	215bp	[10]
rs610932	R:5′ -ATATG GGGCTTGCCTTTATG-3′			5′… CATG↓…3′	C	165, 50 bp	

HpyCH4III, Helicobacter pylori CH4; MluCI, Micrococcus CI; NIaIII, Neisseria Lactamica

Documentation System (Molecular Imager Gel Doc TM XR + Systems with Image Lab TM 2.0 Software, BioRad, USA).

NEB cutter was used to select the restriction enzymes that cleave the amplified sequence containing SNPs (http://tools.neb.com/NEBcutter) [11].

The amplified PCR product for ABCA7 rs3764650 SNP (intronic variant) was 642 bp; it was digested by HpyCH4III restriction enzyme (New England, BioLabs, catalog no #R0618S). The amplified PCR product for Clusterin rs11136000 (intronic variant) SNP was 389 bp; it was digested by MluCI restriction enzyme (New England, BioLabs, catalog no #R0538S). The amplified PCR product for MS4A6A rs610932 SNP (3 Prime UTR Variant) was 215 bp; it was digested by NIaIII restriction enzyme (New England, BioLabs, catalog no #R0125S).

Sequencing

To validate the RFLP results, eight randomly selected samples from the different genotypes examined were validated by purifying the PCR products using QIAquick PCR Purification Kit (Qiagen) (Cat No./ID: 28104) as per the manufacturer's protocol. Then, samples were directly sequenced using Sanger sequencing technique by ABI3730XL sequencer in LGC genomic GmbH, 12459 Berlin/Germany (www.igcgroup.com). ABI Prism Dye terminator sequencing kit (Applied Biosystems) (Cat. # 4337035) was utilized.

APOE genotyping

We used 100 ng of the isolated DNA, 0.2 mM dNTP, 0.4 μM of each primer F (5′-GCGGGCCCCGGCCTGGTACAC-3′) and R (5′-GACGC GGGCACGGCTGTCCAAGGA-3′), 1.5 mM $MgCl_2$, 7% DMSO, and 1 U ThermoDNATaq (EP0402). PCR conditions was as follows: Initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and elongation at 72 °C for 2 min. The final amplicon extension was performed at 72 °C for 5 min. The amplified PCR product was then digested with 10 U of the restriction enzyme Hha1 for 15 min at 37 °C. The digested PCR products were separated on 4% agarose gel at 120 V for 1 h and analyzed [7].

Statistical analysis

Data analysis was done using Microsoft Excel 2010 and SPSS version 24.0 (SPSS IBM., Chicago, IL). Student's t-test was done to compare the means of normally distributed variables between AD patients and normal controls. The distribution of categorical variables between AD patients and controls was determined by χ^2 test or Fisher's exact-test. The level of significance with $p \leq 0.05$ was considered statistically significant; p value ≤0.01 was considered of high significance and p value >0.05 was considered insignificant.

Results

The electrophoretic analysis of the PCR product of ABCA7 rs3764650 SNP demonstrated two bands (490, 152 bp) for TT genotype carriers and four bands (490, 354, 136, 152 bp) for GT genotype carriers (Figure 1). The electrophoretic analysis of the PCR product for CLU rs11136000 SNP exhibited two bands (222, 167 bp) for CC genotype carriers, four bands (222,167, 120, 47 bp) for CT genotype carriers, and three bands (222, 120, 47 bp) for TT genotype carriers; however, due to the small size of the band (47 bp), it could not be observed and diffused on the agarose gel in the CT and TT genotypes (Figure 2). The electrophoretic analysis of the PCR product of MS4A6A rs610932 SNP demonstrated 1 band (215 bp) for AA genotype carriers, two bands (165, 50 bp) for CC genotype carriers and three bands (215, 165, 50 bp) for CA genotype carriers (Figure 3).

Eight selected samples from the different genotypes determined by DNA sequencing were the same as those determined by RFLP analysis. Figure 4(a) demonstrates the partial sequence chromatograms of Clustrin rs11136000, showing the genotype TT, heterozygous type TC, and the genotype CC. Figure 4(b) demonstrates the partial sequence chromatograms of MS4A6A rs610932 showing the genotype AA, heterozygous type AC, and the genotype CC. Figure 4(c) demonstrates the partial sequence

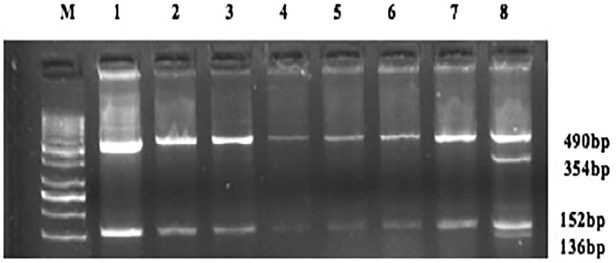

Figure 1: Amplified ABCA7 rs3764650 polymorphism related region was digested with HpyCH4III and run on 3% gel electrophoresis. The gel in the figure shows the different genotypes obtained. M shows the marker. Lane 1–7: shows the TT genotype yielded 490 and 152 bp DNA fragments. Lane 8: shows the GT genotype yielded 490, 354, 136, and 152 bp DNA fragments.

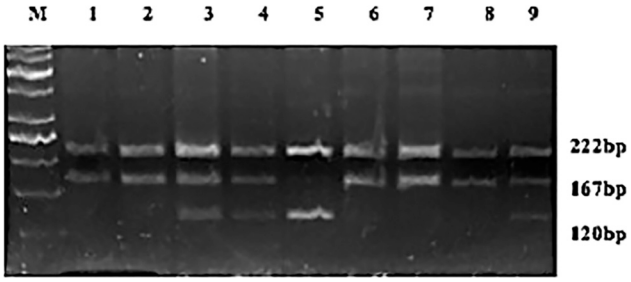

Figure 2: Amplified CLU rs11136000 polymorphism related region was digested with MluCI and run on 3% gel electrophoresis. The gel in the figure shows the different genotypes obtained. M shows the marker. Lane 3, 4, & 9: shows the CT genotype yielded the 222, 167, and 120 bp DNA fragments. Lane 5: shows the TT genotype yielded the 222 and 120 bp DNA fragments. Lane 1, 2, 6, 7, & 8: shows the CC genotype yielded the 222 and 167 bp DNA fragments.

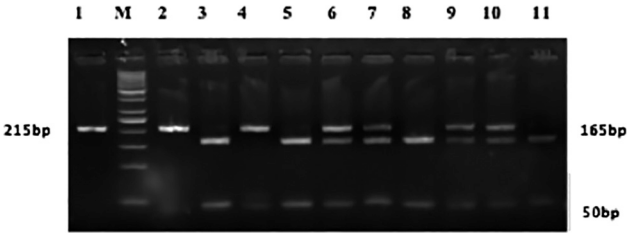

Figure 3: Amplified MS4A6A rs610932 polymorphism related region was digested with NlaIII and run on 3% gel electrophoresis. The gel in the figure shows the different genotypes obtained. M shows the marker. Lane 1, 2, & 4: shows the AA genotype yielded uncut 215 bp DNA fragment. Lane 3, 5, 8, & 11: shows the CC genotype yielded two bands: 165 and 50 bp DNA fragments. Lane 6, 7, 9, &10: shows the AC genotype yielded three bands: 215, 165, and 50 bp DNA fragments.

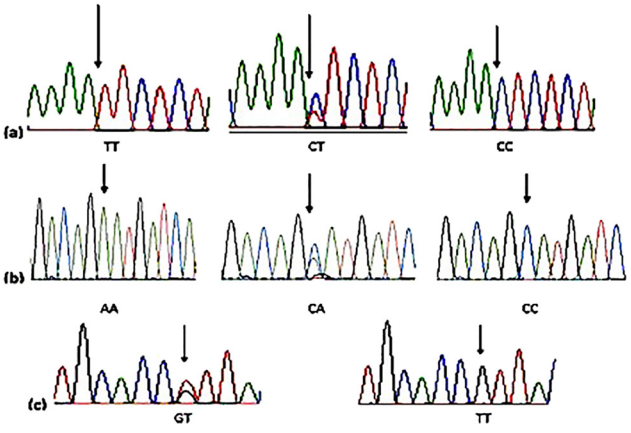

Figure 4: Partial sequence chromatograms shows the different genotypes obtained. (a) CLU rs11136000, the flanking sequence for the SNP: CAAAA [C/T] TCTCTA.(b) MS4A6A rs610932, the flanking sequence for the SNP: AGACAG [A/C] ATGATCA (c) ABCA7 rs3764650, the flanking sequence for the SNP: TTGCACC [G/T] TTACA.

chromatograms of ABCA7 rs3764650 showing the heterozygous genotype GT and the homozygous genotype GG. The allele frequencies and genotype distributions of ABCA7, CLU, and MS4A6A SNPs are shown in (Table 3) for patients and healthy controls.

There was no significant difference between the two studied groups in the genotype distribution and allele frequency of the rs3764650 SNP in ABCA7 gene. The minor allele frequency G was (7%) in AD patients group and (8%) in the control group.

No significant difference was found in genotypic or allelic distribution of CLU rs11136000 between patients and controls. The minor allele frequency T in AD patients and controls was (13%) and 18%, respectively.

No significant difference regarding the genotype distribution and allele frequency of rs610932 SNP in MS4A6A gene was demonstrated between the AD patients and controls where the minor allele frequency C was (42%) and (40%) in AD patients and controls, respectively.

Minor allele frequency (MAF) refers to the frequency at which the second most common allele occurs in a given population. It is widely used in population genetics studies because it provides information to differentiate between common and rare variants in a certain population. MAF of the studied SNPs were checked for the five main worldwide populations reported in the 1,000 Genome project phase 3; the data were imported from dbSNP (release 151) (Figure 5). The MAF (G) for ABCA7 rs3764650 was 8% in the American

Table 3: Comparison between the genotype distributions among the studied subjects (cases & controls) for *ABCA7* rs3764650 SNP, *CLU* rs11136000 SNP, and *MS4A6A* rs610932 SNP.

Genotype	Control group (n=100)	Cases patients (n=100)	p-Value
ABCA7 rs3764650			
GT	16 (16.0%)	14 (14.0%)	0.3
TT	84 (84.0%)	86 (86.0%)	0.7
Allele T	184 (92%)	186 (93%)	0.9
Allele G	16 (8%)	14 (7%)	0.8
CLU rs11136000			
TT	7 (7%)	6 (6%)	0.7
CT	12 (12%)	24 (24%)	0.06
CC	81 (81%)	70 (70%)	0.3
Allele C	174 (87%)	164 (82%)	0.5
Allele T	26 (13%)	36 (18%)	0.2
MS4A6A rs610932			
AA	28 (28.0%)	22 (22.0%)	0.4
AC	63 (63.0%)	72 (72.0%)	0.3
CC	9 (9.0%)	6 (6.0%)	0.4
Allele A	119 (60%)	116 (58%)	0.7
Allele C	81 (40%)	84 (42%)	0.8

Categorical data represented as number and percentage, #, Chi square test; p considered significant if < 0.05.

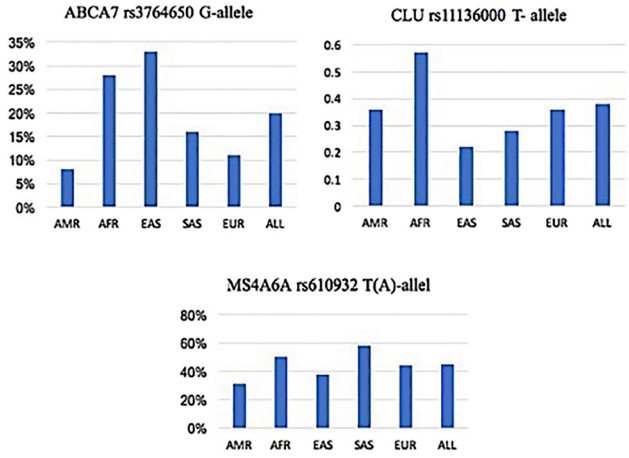

Figure 5: Minor allele frequency (MAF) of the studied polymorphism among population arranged by ethnicity.
Vertical line, MAF of T (A) allele from 1000 genome; horizontal line, ethnicity type. SAS, South Asian; EUR, European; EAS, East Asian; AMR, American; AFR, African.

population which is similar to that in the control group of our analysis. The MAF (T) for CLU rs11136000 was 22% in the East Asian population which is close to that in the of our study (18%). The MAF (C) for MS4A6A rs610932 was 42% in the South Asian population which is similar to that in the AD patients group of our analysis.

The genotypic data of the three evaluated variants was stratified according to the Apo E 4 status in AD patients (Table 4). Apo E 4 carriage was significantly associated with CT genotype of CLU rs11136000 gene (p=0.003). Among AD patients, the frequency of the *CLU* CC genotype was lower in APOE e4 allele carriers APOE e4 allele non-carriers with a significant difference (p=0.003). However, there was no significant association between Apo E 4 and ABCA7 rs3764650 or MS4A6A rs610932 genotypes (p=0.37 and 0.9, respectively).

Gene association networks were analyzed using String online server (http://string-db.org/) to analyze functional interactions [12]. It indicated that the studied genes interact with each other and with other genes (Figure 6). The highest scores of association was for CLU with ApoE and APP (0.953 and 0.987, respectively), while the lowest scores of association was for MSA4A6 with APOE and APP (0.657 and 0.51, respectively). For ABCA7, the scores of association with APOE, CLU, MSA4A6, and APP were 0.726, 0.788, 0.832, and 0.672, respectively. For MSA4A6, the score of association with CLU was 0.7.

Table 4: Correlation of Apo E 4 with different gene polymorphisms in patients with AD.

		Apo E 4 (–) (n=58)		Apo E 4 (+) (n=42)	p-Value
ABCA7 rs3764650 genotype					
GT	6	10.34%	8	19.04%	0.37#
TT	52	89.66%	34	80.96%	
Clustrin rs11136000 genotype					
TT	7	12.06%	2	4.76%	0.003#
CT	4	6.89%	19	45.23%	0.003#
CC	47	81.05%	21	50.01%	
MS4A6A rs610932 genotype					
AA	11	18.96%	9	21.42%	0.9#
AC	43	74.15%	31	73.82%	
CC	4	6.89%	2	4.76%	

Categorical data represented as number and percentage, #, Chi square test; p considered significant if <0.05.

Discussion

Late-onset Alzheimer's disease (LOAD) is a multifactorial neurodegenerative disease coming from complex cooperation among genetic and environmental variables with a high percentage of heritability [13].

The identification of genetic risk factors acting individually or collectively may help in screening for people with elevated risk of cognitive decline such as AD and also for understanding the biological pathways underlying [14].

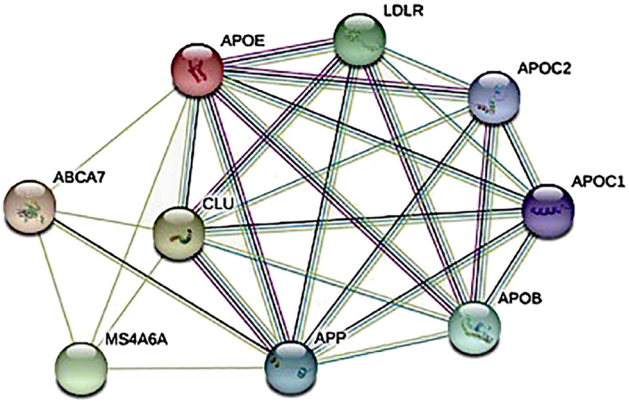

Figure 6: CLU, ABCA7, and MS4A6A gene interactions network obtained from string server with other genes. Protein-protein interaction network and functional module analyses identified the (CLU, ABCA7, MS4A6A and APO E genes) as a highly-interconnected gene and uncovered some evidence of over-representation of "regulation of beta-amyloid formation," "regulation of neurofibrillary tangle assembly," "leukocyte-mediated immunity," and "protein–lipid complex assembly" signaling pathway. APOC1 is assumed to interact with APOE in the lipid metabolism at the cerebral level. Abbreviations: LDLR, low-density lipoprotein receptor; APOB, Apolipoprotein B; APP, amyloid-beta A4 protein; APOC1, Apolipoprotein C-I; APOC2, Apolipoprotein C-II.

Several studies proved the presence of reliable genes which are involved in the risk for LOAD such as APOE4. APOE4 is described to be a significant genetic risk factor for this disease, but alone it is not a sufficient for AD-related brain changes [15]. In a previous study, the percentage of APOE4 allele carriers in Egyptian AD patients was reported to be 28.3% [7].

The lipid carrier ApoE is found in both the central nervous system (expressed mainly in astrocytes and regulated by microglial cells) and the periphery. The risk effects of APOE4 in AD are associated with its pleiotropic functions which influences cholesterol transport, Aβ clearance, and aggregation in the brain, triggering neurotoxicity through tau phosphorylation, neuronal atrophy, synaptic plasticity, microglial function in neurodegenerative diseases, and neuronal inflammation. However, it is important to note that the presence of APOE4 alone is not a sufficient factor for AD-related brain changes such as increased Aβ deposition and decreased glucose metabolism, even in elderly persons. A previous study reported that there is a APOE4 carrier of age 95 years old who has no obvious signs of dementia [16].

Several molecular pathways may have role in the etiology of AD. Major pathways include inflammatory response (ABCA7, CLU and MS4A), lipid metabolism (APOE, CLU, ABCA7, etc.), endocytosis, regulation of cell cycle, and oxidative stress response. Common variants in ABCA7, CLU, MS4A, and other genes have been found to be associated with immune responses for LOAD patients by regulating the clearance of misfolded proteins mediated by glial cells [4].

CLU is an extracellular chaperon which prevents misfolded protein aggregation and promotes the uptake of Aβ deposits by microglial cells for its clearance from the brain. CLU is one of the most associated LOAD risk genes. Previous studies suggested that the CLU mRNA levels were documented to be at higher rates in AD patients. Another study reported that CLU could bind to soluble Aβ peptides to prevent the oligomerization of Aβ plaques. Moreover, the CLU protein level in CSF was found to be significantly increased in patients with AD. CLU can suppress the deposition of Aβ plaques, inhibit the complement system, prevent inflammation, and decrease apoptosis as well as oxidative stress in AD patients [17].

MS4A proteins were found to be involved in the regulation of intracellular Ca^{2+} signaling where the dysregulation of this process has a significant role in AD pathogenesis. Moreover, several members of MS4A cluster have shown the ability to boost the activation of T cells in the brain, thus playing a critical role in AD risk [10].

GWAS of AD and various meta-analysis for different populations have identified multiple loci containing common variant risk alleles such as *ABCA7*, *CLU,* and *MS4A6A* that increase the risk of AD development. Among those loci, *ABCA7* rs3764650 was recognized to be a risk factor for AD, while *CLU* rs11136000 and *MS4A6A* rs610932 were recognized to have a protective role against AD. The influence of SNP on AD prevalence remains a subject of debate and is not the same among various ethnic groups due to the role of gene–environment interaction [18]. Differences in the frequencies of potential risk alleles among different populations were observed. The results of previous studies showed ethnic variations in allelic and genotypic frequencies of these polymorphisms.

A meta-analysis in China studied the three variants investigated in our study and verified the association of *MS4A* rs610932 in the gene cluster with AD susceptibility, but it reported negative results for *CLU* rs11136000 and *ABCA7* rs3764650 [19]. Another updated meta-analysis [3] investigated these variants and observed heterogeneity due to the diverse ethnicities. In addition, this meta-analysis concluded that more case-control studies to understand the involvement of ethnicity in these results are required.

We aimed to examine the association of *ABCA7* rs3764650, *CLU* rs11136000, and *MS4A6A* rs610932 genetic variants with AD in a sample of Egyptian patients and cognitively normal control subjects.

In this study, ABCA7 rs3764650 was found to be consistent with Hardy–Weinberg equation with p value =0.4, while CLU rs11136000 and MS4A6A rs610932 were found to be inconsistent with it as p value were ≤0.05. The human population cannot meet HW assumptions, because there is free migration, health-care for genetic disorders which reduce the effect of natural selection, beside there is no random mating. If the HW is working and effective, then there is no evolution is occurred in the population, but in the real world the evolution had many signs, so, the HW cannot exist in real life.

No significant difference was found regarding the genotype distribution and allele frequency of rs610932 SNP in MS4A6A gene or the rs3764650 SNP in ABCA7 gene. No significant difference was found in genotypic or allelic distribution of CLU rs11136000 between patients and controls.

Apo E 4 carriage was significantly associated with CT genotype of CLU rs11136000 gene and revealed significant difference (p=0.003). Among AD patients, the frequency of the *CLU* CC genotype was lower among APOE e4 allele carriers than non-carriers with a significant difference (p=0.003). Gene interactions could play a critical role in AD etiology and progression. Some studies reported evidence

of two gene-gene interactions that increase AD risk. In a similar study [4], showed a significant association for CC CLU variant in APOE4 negative status and confirmed the modulation of the genetic effect of susceptibility genes for AD by the presence of the APOE 4 allele.

CLU rs11136000 association with LOAD was confirmed in several cohorts [20–23]. However, several studies didn't report this association [24–28]. *MS4A6A* rs610932, according to AlzGene.org, is associated with decreased LOAD risk [29]. This association was confirmed in several cohorts [6, 30]. Previous meta-analyses proved that A allele in rs610932 *MS4A6A* has a protective association with AD [31]. Some other studies [32, 33] failed to show the association between rs610932 and LOAD in different population samples.

The association of ABCA7 rs3764650 genetic variant with LOAD was confirmed in several cohorts [33–35]. However, some studies didn't report this association [36]. A study in Belgian cohort reported other variants in ABCA7 gene that had associations with the risk for LOAD [34]. *ABCA7* SNPs as rs757232 and rs2072102 in another study are reported to have association with higher risk for AD [37]. Observed heterogeneity among various ethnic groups could be due to role of gene–environment interaction.

The limitation of this study was the relatively small sample size. We recommend to confirm this association in a much larger sample of the Egyptian population in future.

In brief, the present findings indicate that the risk for AD has no association with clustrin rs11136000, ABCA7 rs3764650 and MS4A6A rs610932 genes polymorphism. Apo E 4 carriage was significantly associated with CT genotype of CLU rs11136000 gene.

The strength point of the current study is that it the first study to examine the association of ABCA7 rs3764650, CLU rs11136000, and MS4A6A rs610932 polymorphisms with AD in the Egyptian population. Hence, it could be considered as an initiative for further studies using larger cohorts.

Conclusion

No association of CLU rs11136000, MS4A6A rs610932 and ABCA7 rs3764650 with AD was observed. Future genetics studies on AD enrolling a larger number of Egyptian patients from different geographic locations as multi-center national studies are recommended to unmask such complex disorder.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was received from all the subjects included in the study.

Ethical approval: The National Research Centre ethical committee, Cairo, Egypt, revised and approved the research proposal (Ethical Approval Number: 18135) which was in agreement with the Helsinki Declaration of 1975, and as revised in 1983.

References

1. Kang S, Lee Y, Lee JE. Metabolism-centric overview of the pathogenesis of Alzheimer's disease. Yonsei Med J 2017;58:479.
2. Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, Cook MN, et al. Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer's disease: a systematic review and meta-analysis. Neuroepidemiology 2012;38:1–17.
3. Almeida JFF, dos Santos LR, Trancozo M, de Paula F. Updated meta-analysis of BIN1, CR1, MS4A6A, CLU, and ABCA7 variants in Alzheimer's disease. J Mol Neurosci 2018;64:471–7.
4. Moreno DJ, Ruiz S, Ríos Á, Lopera F, Ostos H, Via M, et al. Association of GWAS top genes with late-onset Alzheimer's disease in Colombian population. Am J Alzheimers Dis Other Demen 2017;32:27–35.
5. Nelson AR, Sagare AP, Zlokovic B V. Role of clusterin in the brain vascular clearance of amyloid-β. Proc Natl Acad Sci USA 2017. https://doi.org/10.1073/pnas.1711357114.
6. Proitsi P, Lee SH, Lunnon K, Keohane A, Powell J, Troakes C, et al. Alzheimer's disease susceptibility variants in the MS4A6A gene are associated with altered levels of MS4A6A expression in blood. Neurobiol Aging 2014;35:279–90.
7. Ramadan A, Foda BM, Noha A, Sabry, Refaat O, Saleh AA, et al. Association analysis of ApoE gene polymorphisms among Egyptian patients with Alzheimer's disease. Gene Rep 2019;14: 65–71.
8. Oznur M, Hatipoglu OF, Ayturk Z, Dede S, Akbas K, Aydin D, et al. Association among ABCA7 gene polymorphism, rs3764650 and Alzheimer's disease in the Turkish population. Clin Investig Med 2015;38:E187–91.
9. Ciftci-Yilmaz S, Oznur M, Ayturk Z, Dede S, Cigdem S, Eroglu E, et al. Association of clusterin (CLU) gene polymorphism, Rs11136000, with Alzheimer's disease and diabetes in the Turkish population. Clin Invest Med 2015;38:E200–5.
10. Deng YL, Liu LH, Wang Y, Tang HD, Ren RJ, Xu W, et al. Di the prevalence of CD33 and MS4A6A variant in Chinese Han population with Alzheimer's disease. Hum Genet 2012;131:1245–9.
11. Vincze T, Posfai J, Roberts RJ. NEBcutter: a program to cleave DNA with restriction enzymes. Nucleic Acids Res 2003;31:3688–91. http://www.ncbi.nlm.nih.gov/pubmed/12824395 [Accessed 3 Dec 2018].

12. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015; 43:D447-52.
13. Gatz M, Reynolds CA, Fratiglioni L, Mortimer JA, Berg S, Fiske A, et al. Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 2006;63:168–74.
14. Liao YC, Lee WJ, Hwang JP, Wang YF, Tsai CF, Wang PN, et al. ABCA7 gene and the risk of Alzheimer's disease in Han Chinese in Taiwan. Neurobiol Aging 2014;35. https://doi.org/10.1016/j.neurobiolaging.2014.05.009.
15. Yi D, Lee Y, Byun MS, Lee JH, Ko K, Sohn BK, et al. Synergistic interaction between APOE and family history of Alzheimer's disease on cerebral amyloid deposition and glucose metabolism. Alzheimer's Res Ther 2018;10:84–94.
16. Ulrich JD, Ulland TK, Mahan TE, Nyström S, Nilsson KP, Song WM, et al. ApoE facilitates the microglial response to amyloid plaque pathology. J Exp Med 2018;215:1047–58.
17. Jun G, Naj AC, Beecham GW, Wang LS, Buros J, Gallins PJ, et al. Meta-analysis confirms CR1, CLU, and PICALM as Alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol 2010;67:1473–84.
18. Freudenberg-Hua Y, Li W, Davies P. The role of genetics in advancing precision medicine for Alzheimer's disease—a narrative review. Front Med 2018;5:108–20.
19. Yee A, Tsui NBY, Chang YN, Au CSM, Fok M, Lau LT, et al. Alzheimer's disease: insights for risk evaluation and prevention in the Chinese population and the need for a comprehensive programme in Hong Kong/China. Hong Kong Med J 2018;24: 492–500.
20. Liu G, Wang H, Liu J, Li J, Li H, Ma G, et al. The CLU gene rs11136000 variant is significantly associated with Alzheimer's disease in Caucasian and Asian populations. Neuromolecular Med 2014;16: 52–60.
21. Lancaster TM, Brindley LM, Tansey KE, Sims RC, Mantripragada K, Owen MJ, et al. Alzheimer's disease risk variant in CLU is associated with neural inefficiency in healthy individuals. Alzheimer's Dement 2015;11:1144–52.
22. Du W, Tan J, Xu W, Chen J, Wang L. Association between clusterin gene polymorphism rs11136000 and late–onset alzheimer's disease susceptibility: a review and meta–analysis of case–control studies. Exp Ther Med 2016;12:2915–27.
23. Qiu L, He Y, Tang H, Zhou Y, Wang J, Zhang W, et al. Genetically-mediated grey and white matter alteration in normal elderly individuals with the CLU-C allele gene. Curr Alzheimer Res 2016; 13:1302–10.
24. Sohrabifar N, Gharesouran J, Talebi M, Ghojazadeh M, Mohaddes Ardebili SM. Association of CLU and TLR2 gene polymorphisms with late-onset Alzheimer disease in a Northwestern Iranian population. Turkish J Med Sci 2015;45: 1082–6.
25. Shankarappa BM, Kota LN, Purushottam M, Nagpal K, Mukherjee O, Viswanath B, et al. Effect of CLU and PICALM polymorphisms on AD risk: a study from south India. Asian J Psychiatr 2017;27:7–11.
26. Alaylloğlu M, Gezen-Ak D, Dursun E, Bilgiç C, Hanağasl H, Ertan T, et al. The association between clusterin and APOE polymorphisms and late-onset Alzheimer disease in a Turkish cohort. J Geriatr Psychiatry Neurol 2016;29:221–6.
27. Seripa D, Panza F, Paroni G, D'Onofrio G, Bisceglia P, Gravina C, et al. Role of CLU, PICALM, and TNK1 genotypes in aging with and without Alzheimer's disease. Mol Neurobiol 2018;55:4333–44.
28. Bettens K, Brouwers N, Engelborghs S, Lambert JC, Rogaeva E, Vandenberghe R, et al. Both common variations and rare non-synonymous substitutions and small insertion/deletions in CLU are associated with increased Alzheimer risk. Mol Neurodegener 2012;7:3.
29. Ebbert MTWW, Ridge PG, Wilson AR, Sharp AR, Bailey M, Norton M, et al. Population-based analysis of Alzheimer's disease risk alleles implicates genetic interactions. Biol Psychiatry 2014;75:732–7.
30. Deng YL, Liu LH, Wang Y, Tang HD, Ren RJ, Xu W, et al. The prevalence of CD33 and MS4A6A variant in Chinese Han population with Alzheimer's disease. Hum Genet 2012;131:1245–9.
31. Mao YF, Guo ZY, Pu JL, Chen YX, Zhang BR. Association of CD33 and MS4A cluster variants with Alzheimer's disease in East Asian populations. Neurosci Lett 2015;609:235–9.
32. Wang HZ, Bi R, Hu QX, Xiang Q, Zhang C, Zhang DF, et al. Validating GWAS-identified risk loci for Alzheimer's disease in han Chinese populations. Mol Neurobiol 2016;53:379–90.
33. Stage E, Duran T, Risacher SL, Goukasian N, Do TM, West JD, et al. The effect of the top 20 Alzheimer disease risk genes on gray-matter density and FDG PET brain metabolism. Alzheimers Dement 2016;5:53–66.
34. Cuyvers E, De Roeck A, Van den Bossche T, Van Cauwenberghe C, Bettens K, Vermeulen S, et al. Mutations in ABCA7 in a Belgian cohort of Alzheimer's disease patients: a targeted resequencing study. Lancet Neurol 2015;14:814–22.
35. Vasquez JB, Fardo DW, Estus S. ABCA7 expression is associated with Alzheimer's disease polymorphism and disease status. Neurosci Lett 2013;556:58–62.
36. dos Santos LR, Pimassoni LHS, Sena GGS, Camporez D, Belcavello L, Trancozo M, et al. Validating GWAS variants from microglial genes implicated in Alzheimer's disease. J Mol Neurosci 2017;62:215–21.
37. Allen M, Zou F, Chai HS, Petersen RC, Graff-Radford NR, Dickson DW, et al. Novel late-onset Alzheimer disease loci variants associate with brain gene expression. Neurology 2012; 79:221–8.